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EXAMINER	
HA, JULIE	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/579,641	NELSON ET AL.	
	Examiner	Art Unit	
	JULIE HA	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 September 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 3-32* is/are pending in the application.

4a) Of the above claim(s) *14-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 3-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *5/18/2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Election/Restriction filed on July 16, 2008 and sequence compliance filed on September 15, 2008 is acknowledged. Claim 2 has been cancelled. Claims 1, 3-32 are pending in this application.

Restriction

1. Applicant's election of Group I in the reply filed on July 16, 2008 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

2. The restriction requirement is deemed proper and is made FINAL in this office action. Claims 14-32 are hereby withdrawn from further consideration, as being drawn to nonelected inventions. Claims 1 and 3-13 are examined on the merits in this office action.

3. On telephonic interview on June 16, 2008, the election of species was withdrawn in view of specification.

Objection

4. The specification is objected to for the following minor informality: At page 6, line 6 of the specification, there is a grammatical error. The specification recites, "...general screening method which may used to determine..." This should read, "...general

screening method which may be used to determine..." Applicant is advised to correct this error, and all other possible errors that might be present in the instant specification.

5. The specification is objected to for the following minor informality: At page 8, lines 25-26, there appears to be an error. The specification recites, "...the range 5 to 60 amino acid residues, more preferably in the range 5 to 60 amino acid..." The range 5 to 60 amino acid residues is recited previously, and therefore is not a preferred range. The Applicant is advised to correct this error.

6. The specification is objected to for the following minor informality: At page 9, lines 25-27, there appears to be an error. The specification recites, "Examples of fluorescent proteins include red fluorescent protein and blue, yellow and cyan variants of GFP." It is unclear whether blue, yellow and cyan are variants of GFP or blue, yellow and cyan fluorescent proteins and variants of GFP are separate fluorescent proteins.

Rejection

35 U.S.C. 112, 2nd

7. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 1, 3-13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

9. The base claim 1 recites, "A protein interaction system comprising...a first fluorogenic fragment of fluorogenic fragment of fluorescent protein wherein the fragment

is provided by splitting the fluorescent protein at a site(s) to form complementary fragment such that when complementary fragments of the fluorescent protein are functionally associated with each other, a fluorescent signal capable of being detected is generated…" Claim 1 is further recites, "...a linker portion interposed between the first peptide and first fluorogenic fragment...a second linker portion interposed between the complementary fluorogenic fragment and the second peptide..." The first section of the claim 1 recites that "…when complementary fragments of the fluorescence protein are functionally associated with each other, a fluorescent signal capable of being detected is generated." It is unclear what modifications are included in "functionally associated with each other". Further, it is unclear if the two fragments are functionally associated with each other, a fluorescent signal is generated, how a linker is interposed between the first and the second fluorogenic fragment, this would generate a fluorescent signal. Because claims 3-13 depend from indefinite claim 1 and do not clarify the point of confusion, they must also be rejected under 35 U.S.C. 112, second paragraph.

35 U.S.C. 112, 1st

10. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. Claims 1, 3, 5-13 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The MPEP states that the purpose of the written description requirement is to ensure that the inventor had possession, as of the filing date of the application, of the specific subject matter later claimed by him. The courts have stated:

> "To fulfill the written description requirement, a patent specification must describe an invention and do so in sufficient detail that one skilled in the art can clearly conclude that "the inventor invented the claimed invention." Lockwood v. American Airlines, Inc., 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (1997); *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989) (" [T]he description must clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed."). Thus, an applicant complies with the written description requirement "by describing the invention, with all its claimed limitations, not that which makes it obvious," and by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that set forth the claimed invention." Lockwood, 107 F.3d at 1572, 41 USPQ2d at 1966." Regents of the University of California v. Eli Lilly & Co., 43 USPQ2d 1398.

The MPEP lists factors that can be used to determine if sufficient evidence of possession has been furnished in the disclosure of the Application. These include "level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient." MPEP 2163.

Further, for a broad generic claim, the specification must provide adequate written description to identify the genus of the claim. In Regents of the University of California v. Eli Lilly & Co., the court stated:

"A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials. Fiers, 984 F.2d at 1171, 25 USPQ2d at 1606; *In re Smythe*, 480 F.2d 1376, 1383, 178 USPQ 279, 284-85 (CCPA 1973) ("In other cases, particularly but not necessarily, chemical cases, where there is unpredictability in performance of certain species or subcombinations other than those specifically enumerated, one skilled in the art may be found not to have been placed in possession of a genus. . . ."). Regents of the University of California v. Eli Lilly & Co., 43 USPQ2d 1398.

The MPEP further states that if a biomolecule is described only by a functional characteristic, without any disclosed correlation between function and structure of the sequence, it is "not sufficient characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence." MPEP 2163. The MPEP does state that for generic claim the genus can be adequately described if the disclosure presents a sufficient number of representative species that encompass the genus. MPEP 2163. If the genus has a substantial variance, the disclosure must describe a sufficient variety of species to reflect the variation within that genus. See MPEP 2163. Although the MPEP does not define what constitute a sufficient number of representative, the Courts have indicated what do not constitute a representative number species to adequately describe a broad generic. In Gostelli, the Court determined that the disclosure of two chemical compounds within a subgenus did not describe that subgenus. *In re Gostelli*, 872 F.2d at 1012, 10 USPQ2d at 1618.

In the instant case, the claims are drawn to a protein interaction system comprising a plurality of bait fusion protein, each fusion protein comprising: a first fluorogenic fragment of fluorescent protein wherein the fragment is provided by splitting the fluorescent protein at a site(s) to form complementary fragments such that when complementary fragments of the fluorescent protein are functionally associated with each other, a fluorescent signal capable of being detected is generated, a first peptide

of interest wherein the first peptide of interest of each bait fusion protein is identical to the first peptide of interest in each of the other bait fusion proteins, and a linker portion interposed between the first peptide and first fluorogenic fragment; wherein the linker portions of each bait fusion protein are of different lengths, and the first peptide of interest of each bait fusion protein is identical to the first peptide of interest in each of the other bait fusion proteins, and at least one prey fusion protein comprising a fluorogenic fragment of the fluorescent protein complementary to said first fluorogenic fragment of fluorescent protein, a second peptide of interest, and a second linker portion interposed between the complementary fluorogenic fragment and the second peptide; wherein on interaction of a first peptide of interest with a second peptide of interest, the fluorogenic fragments of the fluorescent protein functionally associate to promote fluorescence. The generic statements bait fusion proteins, fluorogenic fragment of fluorescent protein, a first peptide of interest, a linker protein, prey fusion protein, a second peptide of interest, a second linker do not provide ample written description for the compounds since the claims do not describe a single structural feature. The specification does not clearly define or provide examples of what qualify as compounds of the claimed invention.

As stated earlier, the MPEP states that written description for a genus can be achieved by a representative number of species within a broad generic. It is unquestionable claim 1 is broad generics with respect all possible compounds encompassed by the claims. The possible structural variations are limitless to any class of peptide or a peptide-like molecule that can form peptide or amide bonds, and make

up the class of proteins, linkers and fluorescent protein. It must not be forgotten that the MPEP states that if a peptide is described only by a functional characteristic, without any disclosed correlation between function and structure of the sequence, it is "not sufficient characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence." MPEP 2163. Here, though the claims may recite some functional characteristics, the claims lack written description because there is no disclosure of a correlation between function and structure of the compounds beyond compounds disclosed in the examples in the specification. Moreover, the specification lack sufficient variety of species to reflect this variance in the genus since the specification does not provide any examples of derivatives. The specification is void of organic molecules that functions as a peptide-like molecule that qualify for the functional characteristics claimed as a peptide or a peptide-like molecule or other peptidic molecules, and other synthetic peptide or peptide-like molecule that can form peptide bonds, and function as proteins, linkers and fluorescent proteins.

The specification discloses that fluorescence will only be promoted when peptides of interest of bait and prey fusion proteins, having suitable linker lengths to allow the respective fluorescent protein fragments to associate are used (see paragraph [0021] of instant specification US 2007/0111192 A1). The specification discloses that the provision of a peptide or interest linked to a fluorescent fragment via a range of linker lengths is advantageous over a single linker length as such a range maximizes the chances of an interaction between peptides of interest being detected and minimizes the chances that the fluorescent fragments cannot associate with each other

due to stereochemical hindrance or that the linkers are too flexible (see paragraph [0022] of instant specification as described above). The specification discloses that the linker portions comprise in the range 5 to 60 amino acid residues, more preferably in the range 5 to 60 amino acid, yet more preferably in the range 20 to 60 amino acid residues...at least 20 amino acids (see paragraphs [0027]-[0028] of instant specification as described above). The specification is limited to the linker sequence GGGGS (SEQ ID NO:1) (see paragraph [0033] of instant specification as described above) and multiples of a pentapeptide sequence GGGGS. The specification discloses that such sequences provide advantageous flexibility properties and thus enable the linker region to be readily extended to provide a robust screening method (see paragraph [0056] of instant specification as described above).

The specification discloses that the fluorescent protein is any fluorescent protein in which appropriate split sites can be formed and which the resulting fragments can associate with each other and cause fluorescence may be used...Examples of fluorescent protein include RFP, BFP, YFP, CFP, and variants of GFP (see paragraph [0034] of instant specification described above). The specification discloses that the fragments of fluorescent protein (fluorogenic fragments) are generatable through the introduction of a split point between the amino acids at positions 157 and 158, or between the amino acids at positions 172 and 173 of the humanized form of GFP (SEQ ID NO:2) (see paragraph [0037] of instant specification as described above). Further, the specification discloses that alternate split points are between residues 23/24, 38/39,

50/51, 76/77, 89/90, 102/103, 116/117, 132/133, 142/143, 190/191, 211/212 or 214/215 of EGFP (see paragraph [0040] of instant specification as described above).

The specification discloses that the peptides of interest linked to the fragments of fluorescent protein can be small peptides of differing amino acid sequence, for example nonomers, comprising different amino acid compositions or the same over composition, but with the amino acid present in a different order. Alternatively, the peptides may be full size proteins, e.g. obtained from a cDNA library. Peptides may be produced synthetically or recombinantly using techniques which are widely available in the art. For peptides translated in the cell, naturally or induced, post-translational modification for example glycosylation, lipidation, phosphorylation of the peptides may occur, and these post translated products are still to be regarded as peptides (see paragraph [0048] of instant specification described above).

The working example 1 describes generation of GFP fragments (see paragraphs [0138]-[0142] of instant specification as described above). Example 2 describes the effect of varying the length of the intervening hydrophilic linkers interposed between complementary fragments of fluorescent protein and leucine zipper proteins known to bind to each other (see Example 2). The specification discloses that "in order to ascertain that the haptoEGFP tagged glycoproteins were capable of forming a biologically active complex at the cell membrane cells were transfected with constructs expressing a number of different H and F chimeras…By three days post-transfection, cell to cell fusion was detected over large areas of the monolayer" (see Example 4). The specification does not describe any other bait fusion protein, prey protein, linkers,

fluorogenic fragment and fluorescent protein, such as any other type of peptide or peptide-like molecule, small organic molecules, peptidomimetics, amino acid mimetics that act as peptides, proteins, linkers, fluorogenic fragment and fluorescent proteins. Description of a pentapeptide linker GGGGS and EGFP fluorescent protein, and fragments of EGFP is not sufficient to encompass numerous other fragments of other fluorescent proteins, linkers, bait fusion proteins and prey proteins that belong to the same genus. For example, there are varying lengths, varying amino acid compositions, and numerous distinct qualities that make up the genus. For example, the specification discloses that a linker has from 5 to 60 amino acids. Claim 3 recites that the linker has at least 20 amino acids. For at least 20 amino acid residue linkers, this implies that there are $20^{20} = 1.05 \times 10^{26}$ different possibilities for a 20 residue linkers. For a 60 amino acid residue linker, this implies that there are $60^{20} = 3.7 \times 10^{36}$ different possibilities. For 5 residue linker, there are $5^{20} = 9.5 \times 10^{13}$ different possibilities. Additionally, there are infinite numbers of possible bait fusion and prey proteins, since the only description of these proteins are of their functions. Again, the different possibilities depend on the number of amino acids. Thus, if the bait fusion protein contained 100 amino acid residues, then there are $100^{20} = 1 \times 10^{40}$ different possibilities. Therefore, the number of possible bait fusion proteins and prey proteins are vast. Furthermore, the split points described in the specification is for EGFP only, and not for all other fluorescent proteins. Additionally, the specification does not describe the kind or size of different proteins from the myriad of known proteins the ones that can be fused to GFP without destroying the function of GFP. The claims do

not describe a single structural feature. The specification does not clearly define or provide examples of what qualify as compounds of the claimed invention. Therefore, there is not sufficient amount of examples provided to encompass the numerous characteristics of the whole genus claimed.

The description requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention. See *In re Wilder*, 736 F.2d 1516, 1521, 222 USPQ 369, 372-73 (Fed. Cir. 1984) (affirming rejection because the specification does "little more than outlin[e] goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate"). Accordingly, it is deemed that the specification fails to provide adequate written description for the genus of the claims and does not reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the entire scope of the claimed invention.

12. Claims 1, 3 and 5-13 are rejected are under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph, have been described in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). Among these factors are: (1) the nature or

the invention; (2) the state of the prior art; (3) the relative skill of those in the art; (4) the predictability or unpredictability of the art; (5) the breadth of the claims; (6) the amount of direction or guidance presented; (7) the presence or absence of working examples; and (8) the quantity of experimentation necessary. When the above factors are weighed, it is the examiner's position that one skilled in the art could not practice the invention without undue experimentation.

While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

(1) The nature of the invention and (5) the breadth of the claims:

The claims are drawn to a protein interaction system comprising a plurality of bait fusion protein, each fusion protein comprising: a first fluorogenic fragment of fluorescent protein wherein the fragment is provided by splitting the fluorescent protein at a site(s) to form complementary fragments such that when complementary fragments of the fluorescent protein are functionally associated with each other, a fluorescent signal capable of being detected is generated, a first peptide of interest wherein the first peptide of interest of each bait fusion protein is identical to the first peptide of interest in each of the other bait fusion proteins, and a linker portion interposed between the first peptide and first fluorogenic fragment; wherein the linker portions of each bait fusion protein are of different lengths, and the first peptide of interest of each bait fusion protein is identical to the first peptide of interest in each of the other bait fusion proteins, and at least one prey fusion protein comprising a fluorogenic fragment of the fluorescent

protein complementary to said first fluorogenic fragment of fluorescent protein, a second peptide of interest, and a second linker portion interposed between the complementary fluorogenic fragment and the second peptide; wherein on interaction of a first peptide of interest with a second peptide of interest, the fluorogenic fragments of the fluorescent protein functionally associate to promote fluorescence.

(2) The state of the prior art and (4) the predictability or unpredictability of the art:

With regards to the effect of amino acid substitution in a peptide or protein, the art is unpredictable.

Rudinger (Peptide Hormones, JA Parsons, Ed., 1976, 1-7) teaches that, "The significance of particular amino acids and sequences for different aspects of biological activity cannot be predicted a priori but must be determined from case to case by painstaking experimental study" (see p. 6). Additionally, SIGMA states that with regards to design of peptide sequences that, "Even for relatively short sequences, there are essential and non-essential (or less important) amino acid residues, although the relative importance of the individual amino acid residues is not always easy to determine" (see p. 1). SIGMA further describes what effect some substitutions may have, rather than what effect they will have on hydrophobicity, secondary structure (which will affect tertiary and quaternary structure), and solubility.

With regards to prediction of the native conformation of a protein (structure), the art is unpredictable. Berendsen (Science, 1998, 282: 642-643) states, "The prediction of the native conformation of a protein of known amino acid sequence is one of the great

open questions in molecular biology and one of the most demanding challenges in the new field of bioinformatics" (see p. 642). Furthermore, Berendsen states that "Folding to the stable native state [computationally] has not (yet) occurred, and the simulations do not contain any relevant statistics on the process. The real protein will fold and refold hundreds to thousands of times until it stumbles into the stable conformation with the lowest free energy. Because this hasn't happened (and couldn't happen) in the simulations, we still cannot be sure of the full adequacy of the force field" (see p. 642).

Further, the effects of a single amino acid substitution can have substantial effects on proteins in structure and/or function and are exemplified by the difference between hemoglobin (Hb) and abnormal hemoglobins, such as sickle-cell hemoglobin (HbS). Voet et al teaches that the mutant hemoglobin HbE [GluB8(26)β to Lys] has, "no clinical manifestations in either heterozygotes or homozygotes" (see p. 235). Further, Hb Boston and Hb Milwaukee both have single point mutations which results in altered binding affinity and ineffective transfer from the Fe(III) to Fe(II) oxidation state. Conversely, a single point mutatin in Hb Yakima results in increased oxygen binding by the heme core, and in Hb Kansas, the mutation causes the heme center to remain in the T state upon binding oxygen (rather than structurally rearranging to the R state) (see p. 236). Further, HbS is a single point mutation, Val to GluA3(6)β (see p. 236), which results in deformation and rigidity of the red blood cell. The mutation also provides protection against most malarial strains.

Additionally, the art recognizes that "The significance of particular amino acids and sequences for different aspects of biological activity cannot be predicted a priori but

must be determined from case to case by painstaking experimental study". Additionally, SIGMA states that with regards to design of peptide sequences that, "Even for relatively short sequences, there are essential and non-essential (or less important) amino acid residues, although the relative importance of the individual amino acid residues is not always easy to determine" (see p. 1). SIGMA further describes what effect some substitutions may have, rather than what effect they will have on hydrophobicity, secondary structure (which will affect tertiary and quaternary structure), and solubility. Therefore, any modification on the polypeptide might have an affect on the polypeptide, thus vast numbers of experimentation would be required to see if the polypeptide modified with the oxime-containing non-natural amino acid would have the same affect on certain diseases as the wild-type polypeptide. As with all peptides, activity is based on the structure of the peptide. That is, the peptide has to have the proper structure to recognize the specific receptor for the peptide to be active. The sate of the art for prediction of the native conformation of the protein is, at best, a vague science. For example, in peptide chemistry, Ngo et al teach that for protein and peptides, a "'Direct' approach to structure prediction, that of directly simulating the folding process, is not yet possible because contemporary hardware falls eight to nine orders of magnitude short of the task" (see p. 493). Accordingly, it is not known if an efficient algorithm for predicting the structure exists for a protein or peptide from its amino acid alone (see p. 492). Thus, activity of a given peptide cannot be based on its structure alone. Similarly, the Rudinger article (see the conclusion in particular) states "The significance of particular amino acids or sequences for different aspects of biological activity cannot be

predicted *a priori* but must be determined from the case to case by painstaking experimental study." Finally, in an article published in Science, the author concluded that "one of the 'grand challenges' of high-performance computing-predicting the structure of proteins-acquires much of the flavor of the Holy Grail-quest of the legendary knights of King Arthur. It is extremely desirable to possess but extremely elusive to obtain" (see p. 643 in Berendsen). Berendsen et al states "at the present level of sophistication, [homology modeling] are effective for only 25% of the proteins for which the amino acid sequence is known" (see p. 642). It is known that proteins fold into their native conformation spontaneously and within seconds. The underlying principle of folding is known in the art yet the art lacks the ability to mimic native folding process (see p. 642 in Berendsen). "[E]xisting computers cannot sample enough configurations in a reasonable time to come up with the thermodynamically stable native structure;...we are not too sure that the available force field descriptions, which we need to compute the energy of a each configuration, are accurate enough to come up with reliable free energy of a conformation" (see p. 642 in Berendsen). Berendsen et al discloses the principle of the "Levinthal's paradox" which states that if one was to assume that "three possible states for every flexible dihedral angle in the backbone of a 100 protein residue, the number of possible backbone configuration is 3^{200}. Even an incredibly fast computational or physical sample in 10^{-15}s would mean that complete sample would take 10^{90}s, which excides that age of the universe by more than 60 orders of magnitude." Other tools such as lattice models provide insight into principle of folding, but to provide no solutions to the real folding problems (see p. 643 in

Berendsen). The art has recognized that even single point mutations can cause diverse effects on peptide activity. It has been shown in numerous peptides that a single amino acid can have deleterious effects on the peptide. For example, Bradley et al teach that a single substitution of Ala to Gly in six analogous structural peptides of an ankyrin protein resulted in dramatic and diverse effects on protein stability (see Bradley et al). Sickle cell anemia can be traced to a single point mutation at position six in the beta globulin protein. The instant application claims are open to oxime modification at any position of any therapeutic polypeptides. The working examples given do not sufficiently establish whether any peptide encompassed by the claimed invention would behave similarly. Given that point mutations can lead to abolishment of activity, one would be burdened with undue experimentation to screen the numerous compounds in attempting to find those that have the same activity as the wild-type therapeutic polypeptides.

Furthermore, Hamilton et al (US 2002/0146701) teach that inserts of the short sequence LEEFGS between adjacent residues at 10 internal insertion sites were tried. Of these, inserts at three sites, between residues 157-158, 172-173 and 194-195 gave fluorescence of at least 1% of that of the wild type GFP. Only inserts between residues 157-158 and 172-173 had fluorescence of at least 10% of wild type GFP (see paragraph [0008]). In the same paragraph Hamilton teaches that GFP is a valuable marker for intracellular protein localization. However, the fusion of GFP with structural proteins can alter their properties, resulting in loss of fusion protein localization, decreased GFP fluorescence or both.

Given that one could not determine the structure of a protein computationally, and that the effect of amino acid substitution is unpredictable, it flows logically that one would be unduly burdened with experimentation to determine the effect of amino acid substitution(s) in a peptide or protein, with regards to structure, function, or physical/chemical properties. Therefore, making any peptide having at least 20 amino acid residues as the linker superposed in between proteins and fluorescent fragments that has the same activity as the claimed protein, one would be unduly burdened with experimentation to determine the effect of amino acid content, substitution(s), addition and deletions in a peptide or protein, with regards to structure, function, or physical/chemical properties.

(3) The relative skill of those in the art:

The relative skill of those in the art is high.

(6) The amount of direction or guidance presented and (7) The presence or absence of working examples:

The specification is limited to the peptide or peptide-like molecules that belong to the same class of protein, GFP (EGFP, SEQ ID NO:2), its fragments, and linkers of pentapeptide having the sequence GGGGS (SEQ ID NO:1). The working example 1 describes generation of GFP fragments (see paragraphs [0138]-[0142] of instant specification as described above). Example 2 describes the effect of varying the length of the intervening hydrophilic linkers interposed between complementary

fragments of fluorescent protein and leucine zipper proteins known to bind to each other (see Example 2). The specification discloses that "in order to ascertain that the haptoEGFP tagged glycoproteins were capable of forming a biologically active complex at the cell membrane cells were transfected with constructs expressing a number of different H and F chimeras…By three days post-transfection, cell to cell fusion was detected over large areas of the monolayer" (see Example 4) . The specification does not describe any other peptides linkers that are 5 to 60 amino acid residues in lengths, at least 20 amino acids in lengths, bait fusion protein, prey protein, fluorescent protein fragment, and fluorescent protein, such as synthetic small molecules that are peptide-like molecules that can form peptide bonds, other non-natural amino acids, such as D-amino acids or beta-amino acids, peptidomimetics and amino acid mimetics. Descriptions of EGFP, split points for the EGFP (such as 157/158, 172/173), pentapeptide linkers (GGGGS) are not sufficient to encompass numerous other proteins and molecules that belong to the same genus. However, the specification does not provide for the myriad of peptides embraced by the broad generic or for the myriad of peptides which are embraced by any size linker superposed between the protein and fluorogenic fragment, any fusion protein, any prey protein, and any fluorescent protein. Further, the split points described in the specification is only for the EGFP. Not all fluorescent proteins would have the same split points. Further, Hamilton et al (US 2002/0146701) teach that inserts of the short sequence LEEFGS between adjacent residues at 10 internal insertion sites were tried. Of these, inserts at three sites, between residues 157-158, 172-173 and 194-195 gave fluorescence of at least 1% of

that of the wild type GFP. Only inserts between residues 157-158 and 172-173 had fluorescence of at least 10% of wild type GFP (see paragraph [0008]). Thus, not all split points would function the same. Additionally, the specification does not describe the kind or size of different proteins from the myriad of known proteins the ones that can be fused to GFP/fluorescent protein without destroying the function of GFP or other fluorescent proteins. For example, there are varying lengths, varying amino acid compositions, and numerous distinct qualities that make up the genus. Since there are 20 naturally occurring amino acids, the possibilities are limitless.

(8) The quantity of experimentation necessary:

Considering the state of the art as discussed by the reference above and the high unpredictability and the lack of guidance provided in the specification, one of ordinary skill in the art would be burdened with undue experimentation to make a protein interaction system comprising plurality of bait fusion protein, comprising fluorogenic fragment linked by a linker that would functionally associate each complementary fragment of the fluorogenic fragment to generate a signal capable of being detected. Given that one could not determine the structure of a protein computationally, and that the effect of amino acid substitution is unpredictable, it flows logically that one would be unduly burdened with experimentation to determine the effect of amino acid substitution(s) in a peptide or protein, with regards to structure, function, or physical/chemical properties. Therefore, making any peptide having at least 20 amino acid residues as the linker superposed in between proteins and fluorescent

fragments that has the same activity as the claimed protein, one would be unduly burdened with experimentation to determine the effect of amino acid content, substitution(s), addition and deletions in a peptide or protein, with regards to structure, function, or physical/chemical properties.

35 U.S.C. 102

13. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

14. Claims 1, 8-11 and 13 are rejected under 35 U.S.C. 102(b) as being anticipated by Hu et al (Molecular Cell, April 2002, 9: 789-298, filed with IDS).

15. Hu et al teach a composition comprising proteins fused to different fragments of yellow fluorescent proteins (YFP) connected by linker peptides of different lengths (for example, KQKVMNH and RSIAT) (see p. 797, left column, "Experimental Procedures"). The reference further teaches a protein of interest and two different linkers interposed between the YFP and the protein of interest (see page 797, left hand column). The reference teaches that green fluorescent proteins forms through an autocatalytic cyclization reaction subsequent to protein folding (see p. 790, left column, 2nd paragraph). The Experimental section teaches that bZIP domains and EYGP fragments (enhanced Yellow fluorescent protein) were connected by linker peptides (KQKVMNH and RSIAT) and were fused to amino-terminal hexahistidine purification tags. The fusion

proteins were expressed in E. coli and purified using nickel chelate affinity chromatography (see p. 797, left column, first full paragraph). Further, the reference teaches that hexahistidine-tagged proteins containing the bZIP domains of Jun and Fos fused to the N- and C-terminal EYFP fragments as well as bZIP Jun fused to full-length EYFP (see p. 797, left column, 3rd full paragraph). The reference anticipates claims 1, 8-11 and 13.

16. Claims 1 and 5-13 are rejected under 35 U.S.C. 102(b) as being anticipated by Hamilton et al (US 2002/0146701 A1).

17. Hamilton et al teach a method of reconstituting, folding, or reassembling peptides or other binding pairs into a functionally active protein or other complexes using an antiparallel leucine zipper. The reference teaches assays using fusion proteins comprising GFP fragment and test polypeptides for investigating protein-protein interactions (see abstract). The reference teaches a pair of helices, NZ and CZ capable of forming an antiparallel leucine zipper designed to fuse to the dissected GFP fragments via linkers to form NZGFP (N-terminal GFP) and CZGFP (C-terminal) (see paragraph [0061]). The reference claims a protein complex comprising a first and second peptide, each of said peptides being joined to a heterlogous helical domain, said helical domains being noncovalently associated to form an antiparallel leucine zipper, wherein said peptides form a signaling moiety while complexed, wherein said first and second peptides are joined to said helical domains via a linker, wherein each of the first and second peptides comprises a distinct portion of green fluorescent protein (GFP)

(see claims 1-4). The reference teaches that inserts of short sequence LEEFGS between adjacent residues at 10 internal insertion sites were tried. Of these, inserts at three sites, between residues 157-158, 172-173 and 194-195 gave fluorescence of at least 1% of that of wild type GFP. Only inserts between residues 157-158 and 172-173 had fluorescence of at least 10% of wild type GFP (see paragraph [0008]). The reference teaches that inserts of short sequence LEEFGS between adjacent residues at 10 internal insertion sites were tried. Of these, inserts at three sites, between residues 157-158, 172-173 and 194-195 gave fluorescence of at least 1% of that of wild type GFP. Only inserts between residues 157-158 and 172-173 had fluorescence of at least 10% of wild type GFP (see paragraph [0008]). Furthermore, the reference teaches that the first and second peptides are distinct peptides...derived from GFP, such that they comprise different GFP fragments (see paragraph [0025]). Additionally, the reference teaches that "as used herein, 'fusion protein' or 'chimeric protein' refers to a hybrid protein, which consists of two or more proteins, or fragments thereof, linked together covalently. The reference further teaches a cell based (E. coli) protein interaction system (see for example, Figure 5A-C). A fusion protein may comprise two or more peptides or proteins from different animals, origins, or species (see paragraph [0046]). Therefore, the reference anticipates, claims 1 and 5-13.

35 U.S.C. 103

18. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

19. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

20. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

21. Claims 1 and 3-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hu et al in view of Hamilton et al (2002/0146701 A1) as evidenced by http://www.biovision.com/updated/egfp.html.

22. Hu et al teach a composition comprising proteins fused to different fragments of yellow fluorescent proteins (YFP) connected by linker peptides of different lengths (for example, KQKVMNH and RSIAT) (see p. 797, left column, "Experimental Procedures"). The reference further teaches a protein of interest and two different linkers interposed between the YFP and the protein of interest (see page 797, left hand column). The reference teaches that green fluorescent proteins forms through an autocatalytic cyclization reaction subsequent to protein folding (see p. 790, left column, 2nd paragraph). The Experimental section teaches that bZIP domains and EYGP fragments (enhanced Yellow fluorescent protein) were connected by linker peptides (KQKVMNH and RSIAT) and were fused to amino-terminal hexahistidine purification tags. The fusion proteins were expressed in E. coli and purified using nickel chelate affinity chromatography (see p. 797, left column, first full paragraph). Further, the reference teaches that hexahistidine-tagged proteins containing the bZIP domains of Jun and Fos fused to the N- and C-terminal EYFP fragments as well as bZIP Jun fused to full-length EYFP (see p. 797, left column, 3rd full paragraph). The difference between the reference and the instant claims is that the reference does not teach EGFP and the split sites of 157/158 and172/173, plurality of prey fusion proteins, and prey fusion proteins having different amino acid sequences.

23. Hamilton et al teach a method of reconstituting, folding, or reassembling peptides or other binding pairs into a functionally active protein or other complexes using an antiparallel leucine zipper. The reference teaches assays using fusion proteins comprising GFP fragment and test polypeptides for investigating protein-protein

interactions (see abstract). The reference teaches a pair of helices, NZ and CZ capable of forming an antiparallel leucine zipper designed to fuse to the dissected GFP fragments via linkers to form NZGFP (N-terminal GFP) and CZGFP (C-terminal) (see paragraph [0061]). The reference claims a protein complex comprising a first and second peptide, each of said peptides being joined to a heterlogous helical domain, said helical domains being noncovalently associated to form an antiparallel leucine zipper, wherein said peptides form a signaling moiety while complexed, wherein said first and second peptides are joined to said helical domains via a linker, wherein each of the first and second peptides comprises a distinct portion of green fluorescent protein (GFP) (see claims 1-4). The reference teaches that inserts of short sequence LEEFGS between adjacent residues at 10 internal insertion sites were tried. Of these, inserts at three sites, between residues 157-158, 172-173 and 194-195 gave fluorescence of at least 1% of that of wild type GFP. Only inserts between residues 157-158 and 172-173 had fluorescence of at least 10% of wild type GFP (see paragraph [0008]). Hamilton teaches that GFP mutants with improved solubility properties at higher temperatures and are able to fluoresce at 37°C…a GFP mutant in which phenylalanine is replaced by a leucine (see paragraph [0058]). As evidenced by http://www.biovision.com/updated/egfp.html, any enhanced fluorescent protein gives off a better signal (fluorescence).

24. Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of the references, since the reference teach the protein interaction system utilizing fluorescent protein fragments and different proteins. One of

ordinary skill in the art would have been motivated to combine the teachings, since Hu teaches both GFP and EYFP fragments and linkers of sizes 5 and 7 for protein interaction system, and Hamilton teaches GFP at split points 157-158, 172-173 and 194-195, 4-6 amino acid residue linker and different proteins for protein interaction system. It would have been obvious to try using enhanced green fluorescent protein, since it gives off a better signal and as indicated by Hamilton, certain GFP mutants had improved solubility properties and are able to fluoresce at 37ºC. Furthermore, it would have been obvious to one of ordinary skill in the art to try different linker lengths for optimal lengths for the fluorescent fragments to reassociate with each other to generate fluorescent signal. One of ordinary skill in the art would have been motivated to try EGFP, since Hu teaches the protein interaction system utilizing EYFP. The MPEP states the following in regards to range: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. *"[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)* (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("*The normal desire of scientists or artisans to improve*

upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); In re Hoeschele, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969) (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this principle, see *Merck & Co. Inc. v. Biocraft Laboratories Inc.,* 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997). There is a reasonable expectation of success, since bZIP proteins and EYFP (fluorescent protein) fragments and heterologous protein interaction systems have been successful, and the use of GFP with bZIP was successful, thus one would at least expect that EGFP would at least have the same effect. Therefore, the different linker lengths and different numbers of prey proteins and amino acid sequences of prey proteins are deemed merely a matter of judicious selection and routine optimization that is well within the purview of skilled artisan.

Conclusion

25. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JULIE HA whose telephone number is (571)272-5982. The examiner can normally be reached on Mon-Thurs, 5:30 AM to 4:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang can be reached on 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/J. H./
Examiner, Art Unit 1654

/Cecilia Tsang/
Supervisory Patent Examiner, Art Unit 1654